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Feasibility of conducting human studies to address bromate risks

Kenneth P. Cantor *

Occupational & Environmental Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, DHHS, 6120 Executive Plaza South, Bethesda, MD 20892, USA

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Abstract

Findings from epidemiologic studies have been important in evaluating risk of exposure to many contaminants in drinking water. In the case of bromate, a byproduct of ozone disinfection of water, it is unlikely that observational studies of populations exposed to bromate in drinking water will be as revealing as studies of other contaminants, unless risks are much higher than predicted from laboratory studies of rodents. Occupational exposure to bromate has occurred in the flour milling and baking industries, as well as in chemical production of potassium bromate, used as a flour additive. The feasibility of a cohort study of bromate-exposed workers should be evaluated by studying the conditions and levels of exposure in these occupational settings. Bromate exposure causes oxidative damage to guanine bases of DNA, producing 8-hydroxy-guanine (8-OH-Gua), which is excised by 8-oxoguanosine glycosylase (OGG1) and excreted in the urine. Polymorphic variants of OGG1 in human populations have been associated with elevated cancer risk. 8-OH-Gua and 8-hydroxy-deoxyguanosine (8-OHdG) have been used as biomarkers of oxidative damage in many human studies, and it would be feasible to employ these indicators in controlled clinical experimental settings to see if exposure to bromate in water at levels close to the maximum contaminant level influences urinary levels of excretion, and if so, to help quantify the level of oxidative damage. Such a study could fill an important data gap by providing human data to help estimate the carcinogenic risk from this exposure.

Keywords: Bromate; Epidemiology; 8-Hydroxydeoxyguanosine; 8-Hydroxyguanine; Occupational studies

1. Introduction

Here, we evaluate the feasibility of conducting human studies of bromate exposure and potential cancer risk in the context of toxicologic and epidemiologic data that serve as background and rationale for such research. The types of studies deemed most feasible, from an economic and logistic standpoint, are described. In recent years, epidemiologic research has played a central role in assessing risk of cancer after elevated exposure to selected drinking water contaminants. Epidemiologic studies of waterborne arsenic in Taiwan, Chile, Argentina, and elsewhere established its human carcinogenicity in the absence of an acceptable animal model (International Agency for Research on Cancer, 2004). Cancer risk associated with exposure to complex mixtures of chlorination byproducts has been studied in human populations, with findings strongly suggesting elevated risk, especially for bladder, colon, and rectal cancers (Cantor, 1997). However, the ability of epidemiologic studies to directly elucidate human risk associated with single chemical species, or families of compounds that comprise the mixtures, is limited due

* Tel.: +1 301 435 4718; fax: +1 301 402 1819.
E-mail address: cantork@nih.gov.

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to common pathways of exposure and high levels of covariance among the individual components. Where predominant exposure and metabolic pathways for different chemical families vary (for example, transdermal absorption/inhalation of the trihalomethanes and ingestion of higher molecular weight, more polar compounds) (Nieuwenhuijsen et al., 2000), it may be possible to detect differences in their respective risks. The potential carcinogenicity of nitrate in drinking water is also receiving epidemiologic attention, motivated by evidence for its partial conversion in saliva to nitrite and subsequent reaction in the gut with commonly occurring secondary amines and amides to create *N*-nitroso compounds, most of which are highly carcinogenic in laboratory models (Lijinsky, 1986). Interest in nitrate is underscored by the endogenous production of *N*-nitrosoproline, a marker of in vivo nitrosation detected in the urine of nitrate-exposed subjects dosed with the amino acid proline (Mirvish et al., 1992). Most epidemiologic investigations of carcinogens in drinking water have been studies of morbidity and/or mortality among exposed and unexposed populations. The experimental demonstration of a biomarker of nitrosation in urine is a departure from these more traditional approaches and can serve as a model for an initial evaluation of ingested bromate in humans through the evaluation of a biomarker of bromate.

2. Feasibility of conducting case-control and cohort studies of bromate-exposed populations

Potassium bromate is a rodent carcinogen and is mutagenic in many in vitro testing systems. In 1999, the International Agency for Research on Cancer (IARC) reviewed potassium bromate and concluded that there was inadequate evidence in humans for its carcinogenicity, but sufficient evidence in experimental animals (International Agency for Research on Cancer, 1999). In consequence, potassium bromate was deemed "possibly carcinogenic to humans (Group 2B)". Several commercial uses of potassium bromate were described, as were areas of human activity, which pose opportunities for exposure. Two scenarios, which provide exposure opportunities, and therefore the possibility of epidemiologic evaluation, involve the use of $KBrO_3$ as a flour additive and the presence of the compound in ozonated drinking water.

2.1. Studies of cancer risk after waterborne exposures

In rats, potassium bromate produces renal tubular tumors and thyroid follicular tumors in male and female animals, and peritoneal mesotheliomas in males (International Agency for Research on Cancer, 1999). In mice, a low incidence of renal tubular tumors in males are induced and in hamsters a marginal increase in renal tubular tumors. There is no human evidence that speaks directly to the carcinogenicity of bromate; human risk estimates are therefore based on extrapolation from rodent findings. Direct evidence from human studies, if available, would be important in filling this data gap. Cancer sites observed in rodent studies are not necessarily predictive of the types of cancer that may be found among humans. Therefore, among the range of epidemiologic study designs that may be feasible, case-control studies should not be considered at this time, since this approach depends on the prior selection of particular health end points. Ecologic (geographic correlation) studies and cohort studies among populations exposed to bromate in drinking water may be possible. However, before such study designs are pursued, it would be helpful to have estimates of the expected risk level based on: (1) data from laboratory studies on the quantitative link between measures of oxidative damage to DNA and DNA precursors and cancer risk; and (2) information on how bromate may affect oxidative damage in humans, as discussed below. Therefore, we do not further address the feasibility of direct studies of human cancer risk among populations exposed to bromate in drinking water.

2.2. Occupational studies

Occupational exposure to bromate has occurred at levels that are likely much higher than occur through drinking water. If risk of cancer is evaluated among highly exposed occupational groups, findings could be used to estimate risk at the much lower exposures in drinking water, after accounting for possible differences in exposure route. If occupational studies of reasonable statistical power do not indicate elevated risk, there would be little merit in directly studying risk at lower levels. Bromate has been used historically (and is currently used in the U.S. and Japan) as a flour additive to improve the volume and crumb structure of baked goods. The 1981–1983 National Occupational Exposure Survey conducted by the National Institute for Occupational Safety and Health (NIOSH) reported that approximately 27,000 workers in the U.S. were potentially exposed to bromate during its production and subsequent use as a flour conditioner (National Institute for Occupational Safety and health, 1998). There are no published data available on details of this occupational exposure to bromate. With further development of this information, a retrospective cohort study of exposed

occupational groups might be considered. Further details on occupational exposures to bromate in the baked goods industry could be gathered, including exposure levels, the numbers of employees exposed, and the duration of such exposures. Additional information, such as detailed occupational histories of exposed worker populations and possibilities for mortality or morbidity and vital status followup, could also be gathered to determine feasibility of conducting a cohort study. A study of cancer risk would require identification of a cohort of at least several thousand persons exposed to various levels of bromate, going back at least 30 years from the present. In addition to occupational exposures, widespread exposure of the general population to bromate through ingestion of baked goods has occurred. While most bromate added to flour is converted to bromide during baking, bromate residue in the final product is frequently detected, albeit at low levels. Testing of baked goods in 1992–1993 and again in 1998–1999 by the U.S. Food and Drug Administration detected bromate. In the UK, surveys of bread in 1989 and 1992, observed a median concentration of 35 μg/kg in all six unwrapped breads analyzed in 1989 and <12 μg/kg in 1992 (Dennis et al., 1994). A study primarily involving exposure via ingestion from baked goods in the general population is not feasible because of: (1) the difficulty in assessing exposures with an acceptable level of precision; (2) relatively low levels of exposure; and (3) the difficulty in identifying a non-exposed group.

3. Feasibility of human studies of 8-hydroxy-2′-deoxyguanosine (8-OHdG), a biomarker of oxidative damage

3.1. In vitro and laboratory studies of potassium bromate exposure and oxidative damage

Extensive evidence supports the hypothesis that exposure to potassium bromate results in oxidative damage, in particular the formation of 8-OHdG adducts in the tissue of rodent kidneys, although other organs may be involved (Kasai et al., 1987; Sai et al., 1994; Ballmaier and Epe, 1995; Umemura et al., 1995, 1998, 2004; Chipman et al., 1998; Murata et al., 2001; Mosesso et al., 2004). The evidence derives from both in vitro and in vivo laboratory studies. Pretreatment of $KBrO_3$-exposed rats with resveratrol fully inhibited the increase of 8-OHdG and the increase was partially prevented by melatonin, alpha-phenyl-*N*-tert-butyl nitrone, and Vitamin E (Cadenas and Barja, 1999). These observations suggest that a human study involving experimental exposure $KBrO_3$ in drinking water could be considered.

3.2. 8-OHdG as a marker of oxidative stress in humans

Before considering how 8-OHdG may be used as a biomarker for exposure to bromate, a brief review of this marker of oxidative damage in humans is warranted (Shigenaga et al., 1989; Loft et al., 1993; Loft and Poulsen, 1998, 2000; Toraason, 1999; Kasai et al., 2001; Mayne, 2003). This compound is also called 8-oxy-7-hydrodeoxyguanosine (8-oxodG). An understanding of how baseline levels of urinary 8-OHdG vary among demographic groups, and how levels are influenced by nutritional, occupational, and environmental exposures, is essential to the appropriate design of a study of bromate exposure. Laboratory and human studies have evaluated levels of 8-OHdG in nuclear DNA and urine under a variety of conditions (Loft and Poulsen, 1996). Possible limitations in these studies include conditions of sample storage and the analytical methodology used (Mayne, 2003). Moreover, levels of 8-OHdG in urine have been reported in several ways, making comparisons between studies challenging (Halliwell, 1999). In spite of study limitations, human data are adequate to conclude that there is substantial inter-individual variability (Kasai et al., 2001), men have higher urinary levels of 8-OHdG than women (Loft et al., 1992, 1993; Proteggente et al., 2002), smokers higher levels than non-smokers (Loft et al., 1992, 1993; Suzuki et al., 1995; Loft and Poulsen, 1996, 1998; Prieme et al., 1998; Kasai et al., 2001) and individuals (and species) with high metabolic rates higher levels than those with slower rates (Adelman et al., 1988; Shigenaga et al., 1989; Loft et al., 1992, 1993). Enhanced cytochrome p450-1A2 (CYP1A2) activity has been linked with an increase in 8-OHdG excretion (Poulsen et al., 1998).

When incorporated into DNA, oxidized guanine (8-OH-Gua) can pair with both cytosine and adenine with almost equal efficiency and thereby induce somatic A:T to C:G and G:C to T:A transversion mutations, which can lead to carcinogenesis. The exact relationship between oxidative stress, as estimated by levels of 8-OHdG in the urine, and direct damage to DNA that could lead to carcinogenesis, is not clear (Cooke et al., 2002).

3.3. Human nutritional studies

8-OHdG has been employed as an indicator of oxidative damage in nutritional studies (Mayne, 2003). Two studies of the effects of individual supplements, including Vitamin E, ascorbic acid, and coenzyme Q, showed little influence on levels of urinary or cellular 8-OHdG (Prieme et al., 1997; Huang et al., 2000). In contrast,

several (but not all) studies of dietary modification (beyond supplements only) have shown reduced levels of 8-OHdG. This was found among persons consuming green tea (Hakim et al., 2003), brussels sprouts (Verhagen et al., 1995), tomato sauce (Bowen et al., 2002), red wine (Perez et al., 2002), and soya hypocotyl tea (Watanabe et al., 2000). However, no changes in 8-OHdG were observed among subjects randomized to receive 600 grams of fruit and vegetables/day (Moller et al., 2003).

3.4. Human occupational & environmental exposures

8-OHdG has also been used as a marker of oxidative stress in occupational and environmental settings. In 1999, Toraason (1999) reviewed 11 occupational health studies that measured urinary levels of 8-OHdG. Exposures included asbestos, azo-dyes, benzene, chromium, coal dust, glassworks, rubber manufacturing, styrene, toluene, and environmental tobacco smoke. In all but one investigation, urinary levels of 8-OHdG were higher among exposed workers than among controls. In three studies, findings were not statistically significant. In studies completed since 1999, increased urinary excretion of 8-OHdG was found in workers exposed to polycyclic aromatic hydrocarbons (PAH) (Nilsson et al., 2004), roofers with coal-tar pitch dust and/or asphalt fume exposures (Toraason et al., 2001), boiler makers exposed to metal fumes and residual oil fly ash (Mukherjee et al., 2004), but not among female dry cleaners exposed to perchloroethylene, as compared with laundry workers (Toraason et al., 2003). Ambient air pollution and 8-OHdG was studied in the Copenhagen region of Denmark (Loft et al., 1999), two cities in Eastern Europe (Singh et al., 2003), and Japan (Suzuki et al., 1995). Urban bus drivers in central Copenhagen had higher levels of urinary 8-OHdG than bus drivers with rural/suburban routes, suggesting an association with ambient air pollutants (Loft et al., 1999). In Prague, Czech Republic, there was no difference in excreted 8-OHdG levels between traffic police exposed and unexposed to high levels of PAH; however, differences were observed between comparable occupational groups in Kosice, Poland, with higher urinary levels among the more highly exposed (Singh et al., 2003). In a small study, Suzuki et al. (1995) compared levels of 8-OHdG in the urine of six male smokers and six male non-smokers, finding a statistically significant difference between the groups, consistent with other observations of smokers noted above (Loft et al., 1992, 1993; Suzuki et al., 1995; Loft and Poulsen, 1996, 1998; Prieme et al., 1998). After a baseline measurement of urinary 8-OHdG, three of the non-smokers were exposed for 4 h to ambient air heavily polluted with vehicle exhaust at an intersection with heavy traffic volume. Urinary levels of 8-OHdG from each of these individuals increased significantly, reaching maximum levels from 12 to 24 h after exposure that were significantly higher than those of smokers. This modest experiment suggests an approach for studying how bromate exposures may effect levels of this biomarker of oxidative stress.

3.5. Human cancer risk and polymorphisms in the DNA repair enzyme hOGG1

When incorporated in DNA, oxidized guanine (8-OHGua) can induce somatic A:T to C:G and G:C to T:A transversion mutations, as noted. Indirect evidence that oxidative damage to guanine bases in DNA increases cancer risk comes from studies of polymorphisms in human 8-oxoguanosine glycosylase (hOGG1) and the observation that polymorphic OGG1 proteins differentially suppress mutagenesis induced by 8-OHdG in vivo (Shinmura and Yokota, 2001; Yamane et al., 2004). A large number of enzymes are involved in the recognition and repair of DNA damage. Among them, OGG1 plays a central role in the repair of DNA with oxidative damage to guanine. OGG1 is the first in a sequence of four enzymes involved in this repair mechanism (Loft and Poulsen, 1996). It is hypothesized that polymorphisms in the gene that codes for OGG1 affect repair efficiency, and thereby modify cancer risk. Oxidative stress is likely associated with elevated cancer risk associated with exposure to cigarette smoke, polycyclic aromatic hydrocarbons, and other factors. To date, most studies of hOGG1 polymorphisms and cancer risk have found positive associations. A recent review reported on 19 epidemiologic studies of hOGG1 polymorphism and risk at eight cancer sites (Weiss et al., 2005). The types of cancer found at elevated risk suggest the types of human cancer that may be implicated. Excess risk of lung cancer was observed in several, but not all, studies that examined hOGG1 polymorphism (Sugimura et al., 1999; Wikman et al., 2000; Le Marchand et al., 2002; Vogel et al., 2004; Lan et al., 2004). Most patients had exposure to PAH through cigarette smoke or fumes from smoky coal, with the polymorphism most frequently associated with excess risk being the OGG1-Ser326Cys variant. A complication is that the proportion of the population with this polymorphism differs substantially among different ethnic groups and that different variants are associated with excess risk for lung cancer of different histolo-

gies. Other cancer sites associated with the Ser326Cys polymorphism of hOGG1 are esophagus (Xing et al., 2001), stomach (Takezaki et al., 2002), prostate (Xu et al., 2002), and the nasopharynx (Cho et al., 2003). Breast cancer was not associated with this variant (Choi et al., 2003; Vogel et al., 2003). The Ser326Cys polymorphism was associated with colon cancer among persons with high meat consumption (Kim et al., 2003). Urinary levels of 8-OH-Gua should be modified among persons with this polymorphism. However, few data address this assumption directly. In one study, DNA repair activity of OGG1 in human lymphocytes of healthy individuals was not dependent on the most common genetic polymorphism of OGG1 (Janssen et al., 2001). The studies on cancer risk and OGG1 suggest that any study of urinary 8-OHdG or 8-OH-Gua as a marker of oxidative DNA damage after bromate exposure must consider the OGG1 genotype of individuals. Of importance to the design of human clinical studies is the observation that the levels of OGG1 were induced in rat kidney by exposure of the animals to potassium bromate (Cadenas and Barja, 1999). Thus, the increase in urinary excretion of 8-OHdG or 8-OH-Gua could be related to increased levels of oxidative DNA damage, increased OGG1 activity, or a combination of both.

Another line of evidence comes from studies of the MTH1 gene that encodes an enzyme that hydrolyzes 8-oxo-GTP in the DNA nuceotide precursor pool to monophosphate, thereby preventing occurrence of transversion mutations. MTH1 knockout mice that lack this gene have higher numbers of tumors in the lungs, livers, and stomachs than wild-type mice (Tsuzuki et al., 2001).

3.6. Possible study designs

It would be feasible to study urinary 8-OHdG and/or 8-OH-Gua levels among a small number of individuals exposed to waterborne bromate found at ambient levels in a clinical or other carefully controlled setting. Genotyping for the hOGG1 Ser326Cys substitution polymorphism should be conducted prior to the study. Twenty subjects should suffice, 10 with and 10 without the polymorphic form of hOGG1. If possible, phenotypic measurements of hOGG1 activity should also be conducted, because of the evidence that hOGG1 is inducible (Habib et al., 2003). Changes in 8-OH-Gua levels after bromate exposure could reflect changes in enzymatic activity in removing 8-OH-Gua, and not the degree of oxidative damage to guanine. Subjects should be restricted to nonsmokers of one sex in a relatively narrow age range to minimize variability of baseline levels and response potential. Two study designs are possible:

(1) Direct exposure: following the general pattern of observations of Suzuki et al. (1995) cited above, volunteer subjects normally served by a public water supply with elevated water bromate levels could be exposed to this water in a clinical setting. Subjects would be asked to consume bromate-free water provided by the researcher for a few days before and after the exposure period. Urinary levels of 8-OHdG and 8-OH-Gua would be measured before, during, and after the exposure period, so that each subject would serve as his/her own non-exposed control. To maximize the possibility of detecting effects, the study area should be selected to have a water system with a level of bromate as close to the maximum contaminant level (10 μg/L) as practicable. Various exposure durations could be tested, preferably in the same individuals to enhance comparability of results. The choice of exposure duration would be based on the literature and a range-finding trial. In the Suzuki study (Suzuki et al., 1995), an increase in urinary 8-OHdG was observed after 4 h exposure to motor vehicle exhaust. This suggests that an exposure period of a few hours might suffice, with urine collected before and during exposure, and for 60 h following.
(2) Crossover design: the crossover design has long been used to test pharmaceutical drugs, dietary interventions, and a variety of other exposures (Rothman and Greenland, 1998). Each subject receives both interventions (in this case, water with and without bromate) in a randomized sequence. Adequate time is incorporated between ‘exposures’ so that the effect of one will not influence response to the other. Strengths and weaknesses of the approach are well understood, as are statistical analysis methods (Hills and Armitage, 1979). In a crossover study of bromate exposure, the exposure scenario would be generally as outlined above, that is, volunteer subjects would be exposed to normal levels of bromate in drinking water, and 8-OHdG (and 8-OH-Gua) levels in their urine would be measured. However, here, one-half the subjects would commence the trial with the exposure and then be switched to a non-exposed status and the remaining subjects would follow a reverse sequence.

An investigation of 8-OH-Gua in urine after exposure to bromate under carefully controlled conditions may not yield a positive result. The study involves measurement of a biomarker of oxidative stress after bromate expo-

sure among subjects with other endogenous and possibly exogenous stressors that can lead to the same outcome, and it is unknown whether the magnitude of the proposed bromate effect signal (if any) will be adequately strong to permit observation above normal 'noise'. Using individual study subjects as their own unexposed comparison controls for inter-individual variability increases the possibility of detecting an effect. If there is such an effect, it will not be known absent such an investigation, and other valuable information would be developed during the study, such as the relation between hOGG1 genotype and urinary 8-OHdG or 8-OH-Gua.

4. Summary

To study the carcinogenic effects of bromate in humans, at least two approaches should be considered. Bromate has been used industrially, especially in the baking industry, and a retrospective cohort study of cancer incidence or mortality among exposed workers may be possible. The identification of such exposed populations and the circumstances of their exposure is required to determine whether or not a full-scale effort would be feasible. A second approach would be to examine urinary 8-OHdG and levels of 8-OH-Gua among a small number of volunteer subjects, under controlled conditions, after a challenge with bromate in drinking water. Such information could be used in concert with rodent data of bromate-induced cancer risk in relation to measured levels of DNA damage, as assessed by 8-OHdG and 8-OH-Gua levels. The combined data could lead to estimates of cancer risk in humans at this exposure level. This information would start to fill a major data gap in our understanding of bromate as a potential human carcinogen.

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